

**1. What do you think about microbial NPK biosensor? Limitations?**

**Advantages? Practical? Is it needed?**

He concerns

- i. if we have considered the conditions of the bacterial cells
  - Bacteria will change if you carry them around.
- ii. how quantitative results are produced;
  - Using bio probe is good in general but the limitation is quantitation. It's difficult to make biological system quantitative. How to make biological system quantitative is a question people usually have and feel confused about because the linear range is so wide.
- iii. whether we can detect various concentrations instead of producing a signal when the threshold is reached;
- iv. if the biosensor only allows the detection of low concentrations of NPK; at that point, plant may have suffered from deficiency diseases or died, then is it necessary to detect as we can actually "see" the deficiency?
  - In your case, your device is looking for deficiency of certain nutrient. But when the plant is deficient in a kind of nutrient, there are already some symptoms that can be observed. Will it be too late to mend the situation? The plant may die before the detection due to a lack of NPK. It may not help the agriculture instantly.
- v. if the biosensor can detect the "usual" or "normal" concentrations of NPK in healthy soil;
- vi. if physical and chemical analytical methods are more advantageous as it is accurate, robust, fast and specific than biological analysis, then is it necessary to have biosensing in reality?
  - Sometimes chemical and physical tests are better than relying on biological tests. It does not involve life process and thereby being less

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complicated

- Biological system VS chemical and physical tests:
- Biological system is seldom specific.
- Response is not faster than the physical test.
- Sensitivity is not very high

vii. Safety

- People have hesitation towards transgenic things. You must not release the genetically engineered bacteria to the environment because they contain antibiotic resistant gene. How to contain the bacteria?

**2. When do we apply biosensing?**

Biosensing is much useful for tracking *in vivo* molecular localisation and movement.

**3. Is it possible to apply our biosensor in field?**

Concerning the design of in-field testing, he believes it will be very complicated because we have to consider

- (i) how NPK are directed to the bacterial cells;
- (ii) whether NPK diffuse out or are transported out to the environment after entering the cells;
- (iii) how bacterial cells are kept alive at its optimal condition for a very long period of time;
- (iv) if the bacterial cells are in dormant or active - if it's in dormant, how long should it take to re-activate it?
- (v) how the bacterial cells are safely contained.

**4. Is it enough to test for NPK to ensure the plant grows healthily?**

NPK are macronutrients so plants and crops need a large amount of them to sustain their lives. Micronutrients are also important for the growth of the plants but is need. If a plant is deficient in iron and zinc, it will not be healthy. NPK is the major composition of the plant. For the farmers, they will concern NPK concentrations first.

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If your device can precisely detect the concentrations of NPK, it can help save fertilizer.

- 5. Compounds triggers biochemical pathways intracellularly, do you think it is an effective way of measurement?**

Nope.

- 6. Do you think the microbial biosensor is a threat to safety?**

You never know the risk but it is dangerous. You need to do risk assessment first. We seldom release genetically modified microorganisms to the environment because they contain antibiotic resistant gene. Ampicillin is one of the common antibiotics people use nowadays.

Gene transfer is very likely and rapid between bacteria in the soil.

Food safety brings much concern to farmers and to the government.

- 7. Is it possible to attach bacteria onto a paper to test for the concentrations of NPK?**

But the bacteria may not grow. How can it make GFP for you? Your paper is dry. If yes, you may need to wait for the growth of the bacteria but maybe the environment does not contain the food needed for the growth of the bacteria. You don't know when the bacteria will grow may think of adding soil to your device

- 8. Will it be too late to mend when if the biosensors sense deficiency of certain nutrients?**

Plants usually have tolerance. If they run out of certain kind of nutrients, they may grow up slowly and there is lower yield. The plant may also have storage. If you can detect it earlier

- 9. Can microbial NPK biosensor help organic farmers?**

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Not useful to them. They want to know concentrations of organic nitrogen and carbon. What your biosensor detect is the inorganic compounds but not organic compounds.

**10. Do you think our iGEM project worth a while to continue?**

It is an interesting but it may not be applicable in the market. It is still worth to try as it is a good experience to have a taste of applying knowledge about biotechnology.

**11. How do you monitor the plant growth and soil quality?**

We carry out standard fertilization process. The experienced farmers in China determines both qualities by observation can point out the deficiency of which macro- or micro-nutrient(s).

**12. What is ionomics?**

Profile of ion distribution. Many people who study soil science will study ion distribution through some physical and chemical means e.g. heavy metal

**13. Is microbial biosensor applicable for measuring the ion concentrations in soil?**

It is not efficient to make biosensors for studying ion distribution. It is much useful to use X-ray fluorescence imaging to obtain a complete profile of ions in soil or in plants than using microbial biosensor.